Data Evaluation Record on the Toxicity of BAS 183 H (Dicamba) to Fathead Minnow (*Pimephales promelas*), Early Life Cycle

PMRA Submission Number {.....} EPA MRID Number 48718010

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	402518
	OECD Data Point	{.....}
	EPA MRID	48718010
	EPA Guideline	850.1400

Test material: BAS 183 H **Purity:** 92.9%
Common name: Dicamba
Chemical name: IUPAC: 3,6-dichloro-o-anisic acid
CAS: 3,6-dichloro-2-methoxybenzoic acid
CAS No.: 1918-00-9
Synonyms: Dicamba technical, dicamba acid

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Staff Scientist, CSS-Dynamac Corporation
Signature: David A. McEwen
Date: 12/03/12

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Primary Reviewer: Elizabeth Donovan, Biologist
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Date: 9/7/2016

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Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
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Use Site Category	{.....}	[For PMRA]
EPA PC Code	029801	

Date Evaluation Completed: 11-3-2016

CITATION: Salinas, P.E. 2011. BAS 183 H (Dicamba Techn.) - Early Life-Stage Toxicity Test on the Fathead Minnow (*Pimephales promelas*) in a Flow through System. Unpublished study performed by Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany. Laboratory Project No. 50F0267/97E002. Study sponsored by BASF Corporation, Agricultural Products Division, Research Triangle Park, NC. Study initiated April 20, 2011 and completed August 4, 2011.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the toxicity of a pesticide to fish, early life cycle. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

The 33-day chronic toxicity of BAS 183 H (dicamba) to the early life-stage of fathead minnow (*Pimephales promelas*) was studied under flow-through conditions. Fertilized eggs/embryos (100/level, <2.5 hours old) were exposed at nominal concentrations of 0 (control), 0.10, 0.32, 1.0, 3.2, and 10 mg ai/L (adjusted for purity). Mean-measured concentrations were <0.1 (<LOQ, control), 0.10, 0.33, 1.0, 3.0, and 9.9 mg ai/L, respectively. The test system was maintained at 25 ± 1°C and a pH of 7.6 to 8.0. The overall NOAEC and LOAEC were 9.9 and >9.9 mg ai/L, respectively, as there were no treatment-related effects observed upon any parameter, including time to hatch, hatching success, post-hatch larval or juvenile survival, overall survival, clinical signs of toxicity, or growth (length or wet weight).

This study is classified as scientifically sound and does satisfy guideline requirements for an early life stage toxicity study with fish.

Results Synopsis

Test Organism Size/Age (mean Weight or Length): Embryos, <2.5 hours old
Test Type (Flow-through, Static, Static Renewal): Flow-through

NOAEC: 9.9 mg ai/L
LOAEC: >9.9 mg ai/L

Endpoint(s) affected: none

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted following guidelines outlined in the OECD Guidelines for Testing of Chemicals, Guideline 210, *Fish Early-Life Stage Toxicity Test* (1992); U.S. EPA Series 850 – Ecological Effects Test Guidelines (draft), OPPTS No. 850.1400 *Fish Early Life-Stage Test* (1996); and U.S. EPA FIFRA 72-4(a) (1982) under consideration of the Standard Evaluation Procedure, *Fish Early Life-Stage Test* (1986).

There were no notable deviations from U.S. EPA OPPTS 850.1400 guidance.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality claims statements were provided. This study was conducted in compliance with OECD Principles of Good Laboratory Practice and the GLP Principles of the German "Chemikaliengesetz" (Chemicals Act), which meet the USEPA GLP Standards [40 CFR Part 160 (FIFRA) and Part 792 (TSCA)], with the exception that recognized differences exist between the GLP Principles/Standards of OECD and the Principles/Standards of FIFRA and TSCA.

A. MATERIALS:

1. Test Material BAS 183 H (dicamba, technical-grade)

Description: White solid

Lot No./Batch No. : COD-001266

Purity: 92.9%

Stability of compound under test conditions: The stability of dicamba acid under test conditions was verified during the study.

Storage conditions of Test chemicals: Room temperature

Physicochemical properties of dicamba acid.

Parameter	Values	Comments
Water solubility at 25°C	6.6 g/L	
Vapor pressure	Not Reported	
UV absorption	Not Reported	
pKa	Not Reported	
Kow	Not Reported	

(OECD recommends water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

2. Test organism:

Species:	Fathead minnow (*Pimephales promelas*) [*EPA recommends any of several freshwater fish species, including rainbow trout, brook trout, bluegill, fathead minnow, and channel catfish. See Standard Evaluation Procedure for listing of recommended species. OECD recommends rainbow trout, fathead minnows, zebra fish, and ricefish but does not exclude the use of other species.*]
Age /embryonic stage at test initiation:	Embryos, <2.5 hours old [*EPA recommends fish embryos 2 to 24 hours old.*]
Method of collection of the fertilized eggs:	Following spawning, fertilized eggs were gently removed from spawning tiles (stainless steel half-pipes), pooled, and washed several times. The egg stage was confirmed by examination of a representative sample of eggs using a stereo microscope. All eggs were staged prior to commencement of blastodisc cleavage.
Source:	Parental fish were supplied by Osage Catfisheries, Inc., Osage Beach, MO

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: None reported

b. Definitive study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Parental acclimation, if any Period:	Acclimated for at least 14 days prior to egg collection	
Conditions (same as test or not):	Generally same as test	
Feeding (type, source, amount given, frequency):	Commercial fish diet ("Tetramin" Tetra-Werke, Melle, Germany), frequency and quantity not reported.	
Health (any mortality observed):	In the 2 weeks prior to initiation, 1 of 78 fish died.	

Parameter	Details	Remarks *Criteria*
Number of fertilized eggs/embryos in each treatment at test initiation:	100 embryos/treatment level, divided into 25embryos/replicate and 4 replicate aquaria/treatment level.	*Each treatment should include a minimum of 20 embryos per replicate cup and a minimum of 30 fish per treatment for post-hatch exposure (OECD recommends at least 60 eggs, divided between at least 2 replicates)*
Concentration of test material Nominal: Mean-measured:	0 (control), 0.10, 0.32, 1.0, 3.2, and 10 mg ai/L <0.1 (<LOQ, control), 0.10, 0.33, 1.0, 3.0, and 9.9 mg ai/L	Nominal concentrations were spaced by a factor of 3.2. Water samples were collected from alternating replicate test chambers from the control and treatment levels on Days 0, 5, 12, 19, 26, and 33. Recoveries ranged from 85.3 to 116.3% of nominal concentrations. *A minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate should be used.* *- Toxicant concentration should be measured in one tank at each toxicant level every week.* *- One concentration should adversely affect a life stage and one concentration should not affect any life stage.* *OECD recommends that 5 concentrations be spaced by a constant factor not exceeding 3.2; concentrations of test substance in solution should be within ± 20% of the mean measured values.*
Solvent (type, percentage, if used):	None	*The solvent should not exceed 0.1 ml/L in a flow-through system. Recommended solvents include dimethylformamide, triethylene glycol, methanol, acetone, ethanol.* *OECD recommends that the solvent not have an effect on survival nor produce any other adverse effects; concentration should not be greater than 0.1 ml/L.*

Parameter	Details	Remarks *Criteria*
Number of replicates Control: Solvent control: Treated ones:	4 N/A 4/level	*Number of replicates should be 4 per concentration.* *A solvent control should be used in conjunction with a solubilizing agent.*
Test condition Static renewal/flow-through: Type of dilution system for flow through method: Flow rate: Renewal rate for static renewal:	Flow-through Continuous-flow diluter Flow rate was 1.9L/hr which yielded approximately a 5 fold exchange rate per 24 hours N/A	*Intermittent flow proportional diluters or continuous flow serial diluters should be used. EPA recommends that flow rate to larval cups should provide 90% replacement in 8 to 12 hours (OECD recommends 5 test chamber volumes/24 hours). For static-renewal, OECD recommends 2 renewal procedures; either transfer eggs and larvae to new, clean vessels or reatain organisms in vessels and change at least 2/3 test water. A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used.* *Toxicant Mixing:* *1) Mixing chamber is preferred;* *2) Aeration should not be used for mixing;* *3) The test solution should be completely mixed before introduction into the test system;* *4) Flow splitting accuracy should be within 10%.*

Parameter	Details	Remarks *Criteria*
Aeration, if any:	Slight aeration was initiated in all replicates on Day 18 in response to a low dissolved oxygen measurement.	*Dilution water should be aerated to ensure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.*
Duration of the test:	33 days: 5-day hatching period and 28-day post-hatch period	Fulfills OPPTS requirements. *Recommended test duration is 32 days for EPA. OECD recommendations for test duration are species specific and range from 28-60 days.*
Embryo cups, if used Type/material (glass/stainless steel): Size: Fill volume:	Glass 19-cm diameter cylinders with a screened outlet at a height of 6 cm *ca.* 1.7 L	*Recommended embryo cups are 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.*
Test vessel Type/material (glass/stainless steel): Size: Fill volume:	Stainless steel 29 x 21 x 22 cm, with an overflow 15 cm above the base with a stainless steel screen 9 L	*Recommended test vessel is all glass or glass with stainless steel frame.*
Source of dilution water:	Dilution water was aerated, non-chlorinated drinking water from the municipal waterworks of the city of Frankenthal, Germany, additionally purified through a charcoal filter and diluted with deionized water to achieve a hardness of *ca.* 100 mg/L $CaCO_3$.	*Source of dilution water should be natural or reconstituted water; natural water should be sterilized with UV and tested for pesticides, heavy metals, and other possible contaminants.* *OECD accepts any water in which the test species show control survival at least as good as presented in SEP.*

Parameter	Details	Remarks *Criteria*
Water parameters Hardness: pH: Dissolved oxygen: Temperature(s) (record all the temperatures used for different life stages): Photoperiod: Salinity (for marine or estuarine species): Other measurements: Interval of water quality measurements:	 96 to 102 mg/L as $CaCO_3$ 7.6 to 8.0 5.4 to 8.4 mg/L (≥64% ASV) 23.6 to 26.0°C (mean 25.6°C) 16 hours light/8 hours dark (78 to 193 lux) N/A TOC = 0.7 mg/L on Day 31 Spec. conductance = 254-264 µS Acid capacity (K) = 2.36-2.42 mmol/L Temperature was measured daily in alternating replicates of each group and continuously in one replicate of the control group. Dissolved oxygen (all replicates) and pH (alternating replicates) were measured twice weekly. Total hardness was checked in all replicates on Days 0 and 33. Conductivity and acid capacity were determined on Days 0 and 33.	Hardness and pH range requirements are not reported in OPPTS guidance. *Recommended hardness: 40-48 mg/L as $CaCO_3$;* *Recommended pH:* 7.2 to 7.6 *Dissolved Oxygen (DO) should be measured at each concentration at least once a week;* *Freshwater parameters in a control and one concentration should be analyzed once a week.* *Temperature depends upon test species and should not deviate by more than ±2°C from appropriate temperature.* *OECD recommends that DO concentration be between 60 - 90% saturation. As a minimum DO, salinity (if relevant) and temperature should be measured weekly, and pH and hardness at the beginning and end of the test. Temperature should be measured continuously.*
Post-hatch details When the post-hatch period began: Number of hatched eggs (alevins)/ treatment released to the test chamber: On what day, the alevins were released from the incubation cups to the test chamber:	 Hatching occurred during Days 3 to 5 and swim up was complete by Day 6. All surviving larvae were released Day 14	Hatching success was 98% for the controls. *Percentage of embryos that produce live fry should be ≥ 50% in each control; percentage of hatch in any control embryo cup should not be more than 1.6 times that in another control cup.*

Parameter	Details	Remarks *Criteria*
Post-hatch Feeding Start date: Type/source of feed: Amount given: Frequency of feeding:	 Day 6 (end of swim up) Live brine shrimp nauplii (*Artemia* sp.) and fine milled commercial fish diet ("Tetramin") The quantity was increased corresponding to the size of the fish. Fry were fed two to three times daily up to one day prior to termination of exposure.	
Recovery of chemical: Frequency of measurement: LOD: LOQ:	85.3 to 116.3% of nominal Days 0, 5, 12, 19, 26, and 33 Not reported 0.1 mg ai/L	Based on individual sample results.
Positive control {if used, indicate the chemical and concentrations}:	N/A	
Fertilization success study, if any Number of eggs used: On what day the eggs were removed to check the embryonic development:	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Parameters	Details	Remarks *Criteria*
Parameters measured including the sublethal effects/toxicity symptoms:	- Hatch rate (survival Day 0 to hatch) - Larval survival (hatch to Day 6) -Juvenile survival (Days 6 to 33) -Overall survival (Day 0 to 33) - Measurement of growth (length and wet weight) - Observations of other effects or clinical signs	*Recommended parameters measured include:* *- Number of embryos hatched;* *- Time to hatch;* *- Mortality of embryos, larvae, and Juveniles:* *- Time to swim-up (if appropriate);* *- Measurement of growth;* *- Incidence of pathological or Histological effects;* *- Observations of other effects or clinical signs.*
Observation intervals/dates for: Egg mortality: No. of eggs hatched: Mortality of fry (e.g.,alevins): Swim-up behavior: Growth measurements: Embryonic development: Other sublethal effects	 Daily Daily Daily Daily Day 33 Not determined Daily	
Water quality was acceptable (Yes/No)	Yes	
Were raw data included?	Yes	
Other observations, if any	The maximum biomass loading was 0.13 g fish/L.	Determined at the end of the test, based on the mean wet weight of all individual replicates for all test groups.

II. RESULTS AND DISCUSSION

A. MORTALITY:

No treatment-related effect on survival was observed at any level during the study. For all levels (including the control), hatching success averaged 97 to 98%, survival from hatch to the end of swim-up on Day 6 averaged 97 to 100%, and juvenile survival from the end of swim-up to study termination on Day 33 averaged 98 to 100%. Overall survival (Days 0 to 33) averaged 93 to 97% for all levels, and the resultant NOAEC and LOAEC values for all survival endpoints were 9.91 and >9.91 mg ai/L, respectively, based on mean-measured concentrations.

Table 3: Effect of BAS 183 H (dicamba acid) on egg hatching and survival at different life stage of fish.[a]

Treatment (mg ai/L) Mean-measured (nominal) concentrations	Egg hatched/embryo viability			Time to hatch, Cumulative No. hatched		Juvenile-survival on Day 33	
	No. of eggs at study initiation	Hatch/embryo viability		Day 4	Day 5	% of Day 6	Overall % survival
		No.	%				
Negative Control	100	98	98	36	98	100	97
0.10 (0.10)	100	98	98	37	98	98	93
0.33 (0.32)	100	98	98	37	98	100	97
1.0 (1.0)	100	97	97	29	97	100[b]	96
3.0 (3.2)	100	98	98	30	98	99	97
9.9 (10)	100	98	98	32	98	100[b]	97
NOAEC, mg ai/L		9.9		9.9		9.9	9.9
LOAEC, mg ai/L		>9.9		>9.9		>9.9	>9.9
EC_{50}		N/D		N/D		N/D	N/D
Positive control	N/A						

(a) Data were obtained from Tables 1 and 3 (of Appendix A) on pages 35 and 37 of the study report.
(b) One animal was killed by handling

B. SUB-LETHAL TOXICITY AND OTHER CHRONIC EFFECTS:

Time to hatch: Hatching commenced on Day 3 of the study and by Day 5, hatching was complete for all egg chambers. There was no significant difference between the control levels, or any treatment-related differences when all exposure groups were analyzed.

Swim up: Swim up began on Day 5 and concluded on Day 6 in all groups including controls.

Clinical signs of toxicity: No clinical signs of toxicity were observed at any treatment level. The NOAEC and LOAEC for clinical signs of toxicity were 9.9 and >9.9 mg ai/L, respectively, based on mean-measured concentrations.

Table 4: Effect of BAS 183 H (dicamba acid) on growth of juvenile fish.[a]

Treatment (mg ai/L) Mean-measured (nominal) concentrations	Growth-wet weight (mg ± SD)	Growth-length (cm ± SD)
Negative Control	228.8 ± 39.4	2.8 ± 0.2
0.10 (0.10)	225.8 ± 44.8	2.8 ± 0.2
0.33 (0.32)	232.4 ± 47.7	2.9 ± 0.2
1.0 (1.0)	231.6 ± 43.2	2.8 ± 0.1
3.0 (3.2)	237.2 ± 41.7	2.9 ± 0.2
9.9 (10)	230.6 ± 40.8	2.9 ± 0.1
NOAEC, mg ai/L	9.9	9.9
LOAEC, mg ai/L	>9.9	>9.9
EC_{50}	N/D	N/D
Positive control	N/A	

[a] Data were obtained from Table 7 (of Appendix A) on pages 42 to 47 of the study report.

Growth: Total lengths and wet weights were determined for all surviving fish at study termination, and for both endpoints, there were no statistically-significant differences from the control indicated. For all control and treatment groups, mean lengths ranged from 2.8 to 2.9 cm and mean wet weights ranged from 225.8 to 237.2 mg. The NOAEC and LOAEC values for growth (both endpoints) were 9.9 and >9.9 mg ai/L, respectively, based on mean-measured concentrations.

C. REPORTED STATISTICS:

For the embryo, larvae, and juvenile survival, a pairwise comparison was carried out using a one-sided Fisher's Exact test. In these analyses, the statistical unit was the embryo, larva, or fish, respectively. Additionally, the one-sided Wilcoxon test was performed with the replicate as the statistical unit to compare variability between the replicates.

For the body weights and lengths, a two-sided Dunnett's test was used for a simultaneous comparison of several dose groups with the control.

NOAEC: 9.9 mg ai/L
LOAEC: >9.9 mg ai/L

Endpoint(s) affected: none

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer statistically analyzed the endpoints for hatching success, larval survival, juvenile survival, overall survival, wet weight, and length using Toxstat 3.5 statistical software and replicate data. The fish that were killed by handling were omitted from analysis. The data were assessed for normality and homogeneity of variance using Shapiro-Wilk's and Levene's tests, respectively. The wet weight data met all assumptions and was therefore analyzed using ANOVA followed by Dunnett's test. All other statistically analyzed data met the assumption of homogeneity of variance, but not normality, and transformation of the data failed to correct the issue. Therefore, all other statistically analyzed data were analyzed using Steel's many-one rank test. Significant reductions in a parameter relative to the negative control were used to determine the NOAEC and LOAEC values. The NOAEC and LOAEC based on clinical signs of toxicity and time to hatch were visually assessed. All toxicity values are reported in terms of mean measured concentrations.

Hatching success (embryo survival until hatch)
NOAEC: 9.9 mg ai/L
LOAEC: >9.9 mg ai/L

Larval survival (hatch to end of swim-up)
NOAEC: 9.9 mg ai/L
LOAEC: >9.9 mg ai/L

Juvenile survival (swim-up to end of test)
NOAEC: 9.9 mg ai/L
LOAEC: >9.9 mg ai/L

Overall survival
NOAEC: 9.9 mg ai/L
LOAEC: >9.9 mg ai/L

Wet weight
NOAEC: 9.9 mg ai/L
LOAEC: >9.9 mg ai/L

Length
NOAEC: 9.9 mg ai/L
LOAEC: >9.9 mg ai/L

Clinical signs of toxicity
NOAEC: 9.9 mg ai/L
LOAEC: >9.9 mg ai/L

Time to hatch
NOAEC: 9.9 mg ai/L
LOAEC: >9.9 mg ai/L

E. STUDY DEFICIENCIES:

There were no deviations and/or deficiencies from OCSPP guidance affecting the scientific soundness or acceptability of this study.

F. REVIEWER'S COMMENTS:

The reviewer's statistical conclusions were in agreement with those of the study author. Results were provided

in terms of mean-measured concentrations, rounded to two significant figures.

All validity requirements were met. Specifically, the dissolved oxygen content was maintained ≥60% saturation; the water temperature did not differ by more than ± 1.5°C between test chambers or between successive days at any time during the test, and remained within the temperature range specified for fathead minnow (i.e., 25 ± 2°C); concentrations of the test substance were satisfactorily maintained within ±20% of the mean-measured values; and hatching success and post-hatch survival met criteria delineated in guidance (i.e., >66% and 70%, respectively).

The experimental phase of the study was conducted between May 6 and June 8, 2011.

G. CONCLUSIONS:

This study is scientifically sound and is thus acceptable. There were no treatment-related effects on any parameter, including time to hatch, hatching success, larval or juvenile survival, overall survival, or growth (length or wet weight). In addition, there were no treatment-related clinical signs of toxicity.

NOAEC: 9.9 mg ai/L
LOAEC: >9.9 mg ai/L

Endpoint(s) affected: none

III. REFERENCES:

Organization for Economic Cooperation and Development (OECD). 1992. *Test No. 210: Fish, Early-Life Stage Toxicity Test*, OECD Guidelines for the Testing of Chemicals, Section 2: Effects on Biotic Systems, No. 300, OECD Publishing.

U.S. Environmental Protection Agency. 1982. Office of Pesticide Programs (OPP), 72–4(a) Fish Early Life-Stage and Aquatic Invertebrate Life-Cycle Studies (*in* Pesticide Assessment Guidelines, Subdivision E—Hazard Evaluation; Wildlife and Aquatic Organisms), US EPA report 540/09-82-024.

U.S. Environmental Protection Agency. 1996. Office of Prevention, Pesticides and Toxic Substances, Ecological Effects Test Guideline OPPTS 850.1400, Fish Early-Life Stage Toxicity Test, (*Public Draft*), EPA 712-C-96-121.

Rexrode, M. and T.M. Armitage. 1986. Hazard Evaluation Division Standard Evaluation Procedure - Fish Early Life-Stage Test., U.S. Environmental Protection Agency, Office of Pesticide Programs, Hazard Evaluation Div., EPA/540/9-87/138.

Benoit, D. A. 1982. User's guide for conducting life cycle chronic toxicity tests with fathead minnows (*Pimephales promelas*). U.S. Environmental Protection Agency. EPA/600/8-81/011. 17 pp.

U.S. Environmental Protection Agency. 1996. Office of Prevention, Pesticides and Toxic Substances, Ecological Effects Test Guideline OPPTS 850.1000, Special Considerations for Conducting Aquatic Laboratory Studies, *Public Draft*), EPA 712-C-96-113.

DIN (German Industrial Standard) No. 38409.

Dunnett, C.W. 1955. A multiple comparison procedure for comparing several treatments with a control. Amer. Statist. Assoc. 50, 1096 – 1121.

Dunnett, C.W. 1964. New tables for multiple comparisons with a control. Biometrics 20, 482 – 491.

Siegel, S. 1956. Non-parametric statistics for behavioral sciences. McGraw-Hill, New York.

APPENDIX I: OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Hatching success

```
  Title:  Dicamba fathead ELS hatching success
  File:            8010hs              Transform:              NO TRANSFORMATION

                    Shapiro - Wilk's Test for Normality
  ------------------------------------------------------------------------------

       D =   0.0092
       W =   0.7322

       Critical W  = 0.8840 (alpha = 0.01 , N = 24)
                W  = 0.9160 (alpha = 0.05 , N = 24)

  ------------------------------------------------------------------------------

  Data FAIL normality test (alpha = 0.01). Try another transformation.

  Warning   -  The first three homogeneity tests are sensitive to non-
normality
               and should not be performed with this data as is.


  Title:  Dicamba fathead ELS hatching success
  File:            8010hs              Transform:              NO TRANSFORMATION

                    Levene's Test for Homogeneity of Variance

                                 ANOVA Table
  ------------------------------------------------------------------------------

     SOURCE             DF               SS                MS           F
  ------------------------------------------------------------------------------
     Between             5              0.0003           0.0001       1.0000

     Within (Error)     18              0.0012           0.0001
  ------------------------------------------------------------------------------
     Total              23              0.0015
  ------------------------------------------------------------------------------
                                                          (p-value = 0.4457)

     Critical F  = 4.2479  (alpha = 0.01, df = 5,18)
                 = 2.7729  (alpha = 0.05, df = 5,18)

     Since  F < Critical F  FAIL TO REJECT  Ho: All equal (alpha = 0.01)


  Title:  Dicamba fathead ELS hatching success
  File:            8010hs              Transform:              NO TRANSFORMATION

            Steel's Many-One Rank Test       -      Ho: Control<Treatment
  ------------------------------------------------------------------------------
                                  MEAN IN       RANK      CRIT.           SIG
  GROUP    IDENTIFICATION     ORIGINAL UNITS     SUM      VALUE     DF   0.05
```

1	neg control	0.9800				
2	0.10	0.9800	18.00	10.00	4.00	
3	0.33	0.9800	18.00	10.00	4.00	
4	1.0	0.9700	16.00	10.00	4.00	
5	3.0	0.9800	18.00	10.00	4.00	
6	9.9	0.9800	18.00	10.00	4.00	

Critical values are 1 tailed (k = 5)

Larval survival

Title: Dicamba fathead ELS larval survival (end of swim up)
File: 8010ls Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 0.0060
W = 0.8142

Critical W = 0.8840 (alpha = 0.01 , N = 24)
W = 0.9160 (alpha = 0.05 , N = 24)

Data FAIL normality test (alpha = 0.01). Try another transformation.

Warning - The first three homogeneity tests are sensitive to non-normality
and should not be performed with this data as is.

Title: Dicamba fathead ELS larval survival (end of swim up)
File: 8010ls Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	0.0003	0.0001	0.2000
Within (Error)	18	0.0060	0.0003	
Total	23	0.0063		

(p-value = 0.9583)

```
    Critical F  = 4.2479  (alpha = 0.01, df = 5,18)
                = 2.7729  (alpha = 0.05, df = 5,18)

    Since  F < Critical F  FAIL TO REJECT  Ho: All equal (alpha = 0.01)

  Title:  Dicamba fathead ELS larval survival (end of swim up)
  File:           8010ls            Transform:            NO TRANSFORMATION

           Steel's Many-One Rank Test       -      Ho: Control<Treatment
  ------------------------------------------------------------------------------
                                 MEAN IN       RANK     CRIT.            SIG
  GROUP    IDENTIFICATION     ORIGINAL UNITS    SUM     VALUE     DF    0.05
  ----- --------------------   -----------   -------   ------   -----   ---
    1            neg control      0.9900
    2                    0.1      0.9700        14.00    10.00    4.00
    3                   0.33      0.9900        18.00    10.00    4.00
    4                    1.0      0.9900        18.00    10.00    4.00
    5                    3.0      1.0000        20.00    10.00    4.00
    6                    9.9      0.9900        18.00    10.00    4.00
  ------------------------------------------------------------------------------
  Critical values are 1 tailed ( k = 5 )
```

Juvenile survival

```
  Title:  Dicamba fathead ELS juvenile survival (end of exposure)
  File:           8010js            Transform:            NO TRANSFORMATION

                   Shapiro - Wilk's Test for Normality
  ------------------------------------------------------------------------------

       D =   0.0028
       W =   0.7713

       Critical W  = 0.8840 (alpha = 0.01 , N = 24)
                 W  = 0.9160 (alpha = 0.05 , N = 24)

  ------------------------------------------------------------------------------

  Data FAIL normality test (alpha = 0.01). Try another transformation.

  Warning   -  The first three homogeneity tests are sensitive to non-
normality
               and should not be performed with this data as is.


  Title:  Dicamba fathead ELS juvenile survival (end of exposure)
  File:           8010js            Transform:            NO TRANSFORMATION

                   Levene's Test for Homogeneity of Variance

                              ANOVA Table
```

SOURCE	DF	SS	MS	F
Between	5	0.0014	0.0003	4.2000
Within (Error)	18	0.0012	0.0001	
Total	23	0.0026		

(p-value = 0.0105)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)
= 2.7729 (alpha = 0.05, df = 5,18)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Dicamba fathead ELS juvenile survival (end of exposure)
File: 8010js Transform: NO TRANSFORMATION

Steel's Many-One Rank Test - Ho: Control<Treatment

GROUP	IDENTIFICATION	MEAN IN ORIGINAL UNITS	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	neg control	1.0000				
2	0.1	0.9800	14.00	10.00	4.00	
3	0.33	1.0000	18.00	10.00	4.00	
4	1.0	1.0000	18.00	10.00	4.00	
5	3.0	0.9900	16.00	10.00	4.00	
6	9.9	1.0000	18.00	10.00	4.00	

Critical values are 1 tailed (k = 5)

Overall survival

Title: Dicamba fathead ELS overall survival
File: 8010os Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 0.0092
W = 0.7661

Critical W = 0.8840 (alpha = 0.01 , N = 24)
W = 0.9160 (alpha = 0.05 , N = 24)

Data FAIL normality test (alpha = 0.01). Try another transformation.

Warning - The first three homogeneity tests are sensitive to non-normality
and should not be performed with this data as is.

Title: Dicamba fathead ELS overall survival
File: 8010os Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	0.0019	0.0004	1.1600
Within (Error)	18	0.0060	0.0003	
Total	23	0.0079		

(p-value = 0.3663)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)
= 2.7729 (alpha = 0.05, df = 5,18)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Dicamba fathead ELS overall survival
File: 8010os Transform: NO TRANSFORMATION

Steel's Many-One Rank Test - Ho: Control<Treatment

GROUP	IDENTIFICATION	MEAN IN ORIGINAL UNITS	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	neg control	0.9700				
2	0.1	0.9300	11.50	10.00	4.00	
3	0.33	0.9700	18.00	10.00	4.00	
4	1.0	0.9600	16.00	10.00	4.00	
5	3.0	0.9700	18.50	10.00	4.00	
6	9.9	0.9700	18.00	10.00	4.00	

Critical values are 1 tailed (k = 5)

Wet weight

Title: Dicamba fathead ELS wet weight
File: 8010w Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

```
--------------------------------------------------------------------------------

     D = 1211.7525
     W =   0.9665

     Critical W  = 0.8840 (alpha = 0.01 , N = 24)
              W  = 0.9160 (alpha = 0.05 , N = 24)

--------------------------------------------------------------------------------

Data PASS normality test (alpha = 0.01). Continue analysis.


Title:  Dicamba fathead ELS wet weight
File:             8010w            Transform:             NO TRANSFORMATION

                  Levene's Test for Homogeneity of Variance

                               ANOVA Table
--------------------------------------------------------------------------------

   SOURCE             DF               SS               MS           F
--------------------------------------------------------------------------------
   Between             5             39.4571           7.8914       0.2609

   Within (Error)     18            544.5225          30.2513
--------------------------------------------------------------------------------
   Total              23            583.9796
--------------------------------------------------------------------------------
                                                          (p-value = 0.9286)

   Critical F  = 4.2479  (alpha = 0.01, df = 5,18)
               = 2.7729  (alpha = 0.05, df = 5,18)

   Since  F < Critical F  FAIL TO REJECT  Ho: All equal (alpha = 0.01)

Title:  Dicamba fathead ELS wet weight
File:             8010w            Transform:             NO TRANSFORMATION

                               ANOVA Table
--------------------------------------------------------------------------------

   SOURCE             DF               SS               MS           F
--------------------------------------------------------------------------------
   Between             5            286.0971          57.2194       0.8500

   Within (Error)     18           1211.7525          67.3196
--------------------------------------------------------------------------------
   Total              23           1497.8496
--------------------------------------------------------------------------------
                                                          (p-value = 0.5325)

   Critical F  = 4.2479  (alpha = 0.01, df = 5,18)
               = 2.7729  (alpha = 0.05, df = 5,18)

   Since  F < Critical F  FAIL TO REJECT  Ho: All equal (alpha = 0.05)
```

Title: Dicamba fathead ELS wet weight
File: 8010w Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	neg control	228.9250	228.9250		
2	0.1	225.8750	225.8750	0.5257	
3	0.33	232.4750	232.4750	-0.6119	
4	1.0	231.7000	231.7000	-0.4783	
5	3.0	237.2000	237.2000	-1.4263	
6	9.9	230.8500	230.8500	-0.3318	

Dunnett critical value = 2.4100 (1 Tailed, alpha = 0.05, df = 5,18)

Title: Dicamba fathead ELS wet weight
File: 8010w Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			
2	0.1	4	13.9821	6.1	3.0500
3	0.33	4	13.9821	6.1	-3.5500
4	1.0	4	13.9821	6.1	-2.7750
5	3.0	4	13.9821	6.1	-8.2750
6	9.9	4	13.9821	6.1	-1.9250

Length

Title: Dicamba fathead ELS length
File: 8010l Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 0.0525

```
      W =   0.8751

      Critical W  = 0.8840 (alpha = 0.01 , N = 24)
               W  = 0.9160 (alpha = 0.05 , N = 24)
```

Data FAIL normality test (alpha = 0.01). Try another transformation.

Warning - The first three homogeneity tests are sensitive to non-normality
and should not be performed with this data as is.

Title: Dicamba fathead ELS length
File: 80101 Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	0.0038	0.0008	0.6000
Within (Error)	18	0.0225	0.0013	
Total	23	0.0263		

(p-value = 0.7006)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)
= 2.7729 (alpha = 0.05, df = 5,18)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Dicamba fathead ELS length
File: 80101 Transform: NO TRANSFORMATION

Steel's Many-One Rank Test - Ho: Control<Treatment

GROUP	IDENTIFICATION	MEAN IN ORIGINAL UNITS	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	neg control	2.8500				
2	0.1	2.8500	18.00	10.00	4.00	
3	0.33	2.8750	20.00	10.00	4.00	
4	1.0	2.8250	16.00	10.00	4.00	
5	3.0	2.8500	18.00	10.00	4.00	
6	9.9	2.8750	20.00	10.00	4.00	

Critical values are 1 tailed (k = 5)